PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/003211	21.03.2005	19.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
EPIGENOMICS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	19
	No:	Claims	1-18,20-22
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	-

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to in this communication:

D1: US 2003/082600 A1 (OLEK ALEXANDER ET AL) 1 May 2003 (2003-05-01)
D2: JU J ET AL: "FLUORESCENCE ENERGY TRANSFER DYE-LABELED PRIMERS FOR DNA SEQUENCINGAND ANALYSIS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 92, May 1995 (1995-05), pages 4347-4351

1 NOVELTY (Article 33(2) PCT)

1.1 A calibration standard for determining base proportions of degenerated bases in DNA, wherein a degenerate base represents at least two different bases in at least two DNA molecules at the same position, wherein said standard is produced by a process comprising the steps of:
- providing at least two DNA molecules being not identical and containing at least two bases of the degenerated base at different positions within at least one DNA molecule; and
-mixing the DNA molecules in unequal ratios thereby obtaining the calibration standard is disclosed in D1 (cf. par.78 and 164).
Therefore, the subject-matter of **independent claims 1 and claim 17 is not novel** over D1 (Article 33(2) PCT).

1.2 D1 furthermore discloses the use of a calibration standard according to claims 1 of the present application for determining base proportions of degenerated bases in DNA (cf. par.78 and 164 to 165).
Therefore, the subject-matter of **independent claim 18 is not novel** over D1 (Article 33(2) PCT).

1.3 D1 discloses also a method of calibration of measurements systems which determine base proportion of degenerated bases in DNA, wherein a degenerated base represents at least two different bases in at least two DNA molecules at the same position, characterized by the use of a calibration standard according to claims 1-16 of the present application comprising the steps of
- performing said measurement system with one or several of said calibration standards at least once,
-determining a calibration curve or function
- performing said measurement system with the sample to be analysed
- comparing the result with those of step 1 and 2 and
- assessing the measurement system performed (cf. par.78 and 164 to 165).
Therefore, the subject-matter of **independent claim 20 is not novel** over D1 (Article 33(2) PCT).

1.4 A method for the production of a calibration standard for determining base proportions of degenerated bases in DNA, a degenerated base representing at least two different bases in at least two DNA molecules at the same position comprising the steps of
- providing at least two DNA molecules being not identical and containing at least two bases of the degenerated base at different positions within at least one DNA molecule; and
-mixing the DNA molecules in unequal ratios thereby obtaining the calibration standard is disclosed in D1 (cf. par.78 and 164).
Therefore, the subject-matter of **independent claim 22 is not novel** over D1 (Article 33(2) PCT).

2 INVENTIVE STEP (Article 33(3) PCT)

2.1 Document D1 is considered to represent the most relevant state of the art for claim 19 in its present form. D1 discloses a method for determining base proportions of degenerated bases in DNA, a degenerated base representing at least two different bases in at least two DNA molecules at the same position by sequencing wherein the calibration standard according to claim 1 is used (cf. par.78, 164 to 165).

2.2 The subject-matter of claim 19 differs from the subject-matter disclosed in closest prior art document D1 in that the sequencing is further defined as comprising the steps of (a) providing trails each containing the DNA, a DNA polymerase, a sequencing primer with a label corresponding to any base moiety, 2'-monodeoxy-NTPs, and 2',3'-dideoxyanalog, whereby the 2'-monodeoxy-NTPs are contained in excess compared to the 2',3'-dideoxyanalog; (b) DNA-dependent extension of the sequencing primer by a DNA polymerase whereby fragments of different length with the dideoxyanalogs at the 3' terminus are obtained; (c) unifying the trails; (d) separating the fragments; and (e) detecting the labels, thereby determining the base proportions of degenerated bases.

2.3 No unexpected technical effect appears to be associated with said differences.

2.4 The technical problem to be solved may therefore be regarded as providing an alternative method of sequencing. The proposed solution is to (a) provide trails each containing the DNA, a DNA polymerase, a sequencing primer with a label corresponding to any base moiety, 2'-monodeoxy-NTPs, and 2',3'-dideoxyanalog, whereby the 2'-monodeoxy-NTPs are contained in excess compared to the 2',3'-dideoxyanalog; (b) DNA-dependent extension of the sequencing primer by a DNA polymerase whereby fragments of different length with the dideoxyanalogs at the 3' terminus are obtained; (c) unify the trails; (d) separate the fragments; and (e) to detect the labels, thereby determining the base proportions of degenerated bases.

2.5 This solution cannot be considered as involving an inventive step for the following reasons:

2.5.1 Sanger sequencing using primers with four different labels is one of the routinely applied sequencing methods in molecular biology (cf. D2, p.4348, col.2, par.3). It comprises all the steps (a) to (e). To sequence the DNA following the Sanger sequencing protocol represents merely one of several straightforward possibilities from which the skilled person would select, without the exercise of inventive skill, in order to solve the problem posed, namely to find an alternative sequencing method.

2.6 Hence, the subject-matter of **independent claim 19 does not involve an inventive**

step (Article 33(3) PCT).

2.7 Dependent claims 2 to 16 and 21 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, as all of the additional features fall within the scope of routine laboratory practise.

Re Item VI
Certain documents cited

Certain published documents

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 2004/051224	17/06/2004	03/12/2003	03/12/2002
WO 2005/075671	18/08/2005	07/02/2005	05/02/2004